The Significance of Mycorrhizal Fungi for the Utilization of Different Sources of Phosphate by *Pinus silvestris* L.

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Introduction

The mycorrhizal association is the closest relation between fungal microorganisms and the roots of higher plants. Together with the specific microflora of the rhizosphere this biocoenosis must be considered as a functional entity which significantly influences the supply of the higher plant with mineral nutrients, water and other substances from the soil. This is especially true for the ectotrophic mycorrhiza which are common in forest trees and the physiological significance of which is somewhat clearer than that of the widespread endotrophic mycorrhiza of the type of Endogone. Although many theories were established to explain the positive effects of ectotrophic myccorrhiza which were repeatedly observed and experimentally proved, a definite elucidation of these facts is still lacking. The interrelationships with the microflora of the rhizosphere, in particular, are obscure. Three hypotheses may be considered important to explain the beneficial role of mycorrhiza: 1. Improved nutrition with mineral salts; 2. Improvement of nitrogen nutrition via decomposition of humus; 3. Supply with growth substances produced by the fungi or procured from the soil.

The first hypothesis mentioned was the subject of much attention and scientific research. Nevertheless, many important problems are far from clear owing to considerable experimental difficulties and the complexity of the problem. Obviously mineral nutrition is not only a question of absorption but also a problem of availability, which is greatly influenced by microbiological processes in the rhizosphere.

Furthermore, the significance of different fungal symbionts for the efficiency of mycorrhiza is still quite obscure. The experiments described below were planned as a contribution to elucidate these questions.

Materials and Methods

Pinus silvestris L. was used as test plant and two fungal symbionts: *Suillus* (*Boletus*) *luteus* (L. ex Fr.) Gray and *Amanita muscaria* (L. ex Fr.) Hooker. The first is strongly bound to pines in nature while the latter is usually an associate of birch and aspen and

in some places only occurs together with pine. We examined the absorption efficiency of labelled orthophosphate (P^{32}) in soluble and poorly soluble forms, and the influence of greater microbiological competition on phosphorus absorption by mycorrhizal and non-mycorrhizal plants.

One-year-old pine seedlings grown in sterilized sand were transferred to clay pots with autoclaved sand and inoculated with pure cultures of the test fungi previously cultivated on peat supplied with nutrient solution according to Moser (1958). Abundant mycorrhiza developed soon after inoculation so that in the following year the seedlings could be used as test material. Labelled phosphate in quantities of 20—50 µc P^{32} per plant was brought on layers of filter paper, laid around the root packs and replaced together with these into the pots. Diffusion of labelled material into the porous potwalls was prevented by a layer of corrugated wax cardboard. Assays of phosphorus uptake were performed in the intact plants with an end window Geiger counter connected with a counting rate meter. For this reason the shoots were placed in frames equipped with a plexiglass front and a back cover of colourless plastic gauze. The measuring spots in the plexiglass wall were covered with thin plastic foil to prevent contamination of the counter window. Measurements were made at several day intervals and corrected for disintegration, absorption by the plastic foil and natural background. At the end of the experiments needle samples were taken quantitatively from the measuring spots so that counting rates could be calculated for equal quantities of dry substance. Soluble phosphorus was used as $NaH_2P^{32}O_4$ together with KH_2PO_4 as carrier, while poorly soluble labelled iron-hydroxylphosphate and apatite were synthesized according to Rathje (1942) and Kurmies (1953). Labelled orthophosphate bound to humus (humus phosphate) was prepared by shaking soluble phosphate with fine humus of a beech stand and subsequent repeated extraction with citrate buffer at pH 3. Experiments with increased microbiological competition were performed by supplying each pot with 4 g. amylum solubile several days before application of soluble phosphate in the usual way. Determinations of inactive phosphorus in the needles were made after incineration in sulphuric acid according to Kuttner and Lichtenstein (1932). In all experiments every batch comprised 5—7 plants with two measuring spots each.

Results and Discussion

The method applied permits continuous measurements of phosphorus absorption over long periods and under approximately natural conditions. Therefore the results are more reliable than those of some other authors who used plants transferred to nutrient solutions. Moreover, the mycorrhiza were in no way injured and normal conditions of growth and respiration were insured. This has an important bearing on transport processes in the plants.

Table 1. Phosphorus uptake of mycorrhizal pines from different P-sources (Values in per cent deviation of nonmycorrhizal controls)

Days	Soluble phosphate		Iron phosphate		Apatite		Humus phosphate
	S. luteus	*A. muscaria*	*S. luteus*	*A. muscaria*	*S. luteus*	*A. muscaria*	*S. luteus*
10	+50	−40	−58	−54	+50	− 5	+100
20	+77	−37	−31	−48	+74	−16	+143
30	+48	−44	+10	−16	+50	+ 9	+172
40	+30	−44	+35	−12	+21	+ 8	+162
50			+78	+ 9	+69	+10	+168

Table 1 summarizes the main results of absorption experiments with phosphates of different solubility. In each form of orthophosphate significant differences between the fungal associates could be observed both of which had developed plenty of mycorrhiza with the pine plants. In comparison with nonmycorrhizal controls *Suillus luteus* always stimulated phosphorus absorption distinctly, while in experiments with soluble phosphate *Amanita muscaria* reduced absorption. This could not be overcome even during long lasting experiments (up to 100 days). With iron phosphate and appatite this fungus reached or slightly exceeded the values of the controls, but *Suillus luteus* clearly enhanced absorption in both cases. Absorption from humus phosphate was only tested with the latter fungus.

The experiments yield some remarkable conclusions. Both fungi caused a great enlargement of the surface area by formation of mycorrhiza. Nevertheless, only one species (*Suillus luteus*) was able to increase phosphorus absorption greatly while *Amanita muscaria* in part even diminished absorption. Therefore, the often cited enlargement of surface area cannot be considered the sole cause of the beneficial effect of mycorrhiza. Probably the metabolic efficiency of the fungal symbionts and the carbohydrate supply play the decisive role. This will be true especially for the uptake of orthosphate because absorption of this ion is bound to metabolic phosphorylations. The experiments demonstrated an improved absorption efficiency for some poorly soluble phosphates, especially by *Suillus luteus*. It must be expected, however, that strong acid producers of the fungal microflora of soils are more active in utilizing apatite, in particulary, and other calcium phosphates than mycorrhizal fungi (Meyer and König, 1960) which in general show only weak acid production so that their utilization efficiency is relatively low.

An observation of remarkable interest are significant differences between mycorrhizal and nonmycorrhizal pines grown for two years in clay pots. While the mycorrhizal plants were distinctly more vital and vigorous the nonmycorrhizal controls showed a pale colour of needles and smaller dry weights. The phosphorus content of the needles also gave significant differences, but no difference betwen the two fungal

associates could be demonstrated (Table 2). As the sand used was rather poor in phosphorus the heightened phosphorus levels can hardly be explained by a more intense utilization of the soil material. It may be supposed that a mobilization of mineral salts in the pot walls was initiated by the fungi whose hyphae can easily penetrate the clay material. Under these conditions mycorrhizal hyphae are obviously more

Table 2. Dry weight, shoot length and phosphorus content of needles of mycorrhizal and nonmycorrhizal pines (Mean values of at least 25 plants two years after inoculation)

	Control	*Suillus luteus*	*Amanita muscaria*
Dry weight of plants (g.)	5.77 ± 0.34	9.23*** ± 0.43	9.09*** ± 0.29
Shoot length (cm.)	15.2 ± 0.4	20.1*** ± 0.6	19.6*** ± 0.5
Phosphorus content (µg. P in 100 mg. dry weight)	95 ± 2.3	110** ± 3.0	107* ± 3.9

*** significant at P = 0.001.
** significant at P = 0.01.
* significant at P = 0.05.

effective than nonmycorrhizal roots which lack this ability. Situations of this kind may occur frequently in the soil so that colonization and utilization of microareas must be suspected to be highly important for the mineral nutrition of mycorrhizal plants.

Another problem not yet elucidated are the interrelationships between the rhizosphere flora and mycorrhiza. Katznelson et al. (1963) found the number of microorganisms of mycorrhiza of *Betula lutea* to be about 100 times greater than the population of nonmycorrhizal roots. The number of bacteria, in particular, was greatly increased. This more active rhizosphere population may result in an increased mobilization of nutrients, on the other hand, a strengthened fixation of mineral coumpounds may occur. We investigated, therefore, whether under conditions of activated microbiological competition, mycorrhizal roots behaved differently from nonmycorrhizal controls. The plants were given an additional dose of carbohydrate as soluble starch (amylum solubile) in the rooting area and some days later soluble orthophosphate. Control series were run without the application of carbohydrate. In each batch the mycorrhizal plants absorbed more phosphorus than the controls without mycorrhiza (Table 3). The added starch caused an intense and continuous depression of phosphorus uptake in both mycorrhizal and non- mycorrhizal plants which is explained by the intensified phosphorus fixation by soil microorganisms. It must be concluded that under these conditions at least *Suillus luteus* cannot compete successfully with the activated free living microflora (Table 4). Santoro and Casida (1962) demonstrated

the formation of antibiotics by mycorrhizal fungi, amongst others by *Suillus luteus*. But it is still unkown to what degree and under what circumstances antibiotics are produced in the natural habitat. The efficiency spectra of these substances too have hardly been investigated. It is highly probable, however, that particular species have different potencies in antibiotic formation and in this way certainly influence the absorption of nutrients by the plant. Further investigations in this direction and with

Table 3. Uptake of soluble phosphate by mycorrhizal pines under conditions of artificially stimulated microbiological competition induced by an addition of starch (Values in per cent deviation of nonmycorrhizal controls)

Days	Starch added		Without starch	
	S. luteus	*A. muscaria*	*S. luteus*	*A. muscaria*
10	+151	+50	+150	−40
20	+148	+41	+153	−37
30	+140	+21	+147	−44
40	+136	+15	+167	−44
50	+130	+19	+160	–

Table 4. Depression of phosphorus uptake of mycorrhizal and nonmmycorrhizal pines with and without added starch (Values in c/min. = counts per minute per 100 mg dry weight)

Days	*Suillus luteus*				Control			
			Starch added				Starch added	
	c/min.	%	c/min.	%	c/min.	%	c/min.	%
10	4360	100	1315	30.2	1740	100	524	30.1
20	5700	100	2050	36.0	2250	100	800	35.6
30	6500	100	2350	36.2	2550	100	985	38.6
40	7250	100	2650	36.5	2750	100	1135	41.3
50	7750	100	2900	37.4	2850	100	1250	44.0

some more fungal species are planned. The addition of toxic compounds such as p-chloro-mercuribenzoic acid (blocker of SH-groups) or pentachlorphenol (uncoupling toxin) into the rooting area of the plants caused a depression of phosphorus uptake. It could be shown in one experiment that non-mycorrhizal plants recovered even better from the poisons added. They subsequently increased phosphorus absorption which is obviously due to an injury to the microflora.

It may be concluded that mycorrhizal fungi and also free living soil microorganisms absorb poisons better and are therefore more injured by some toxins than non-mycorrhizal roots. More detailed experiments along these lines and with other fungicides are in preparation.

Summary

1. The efficiency of the mycorrhizal fungi *Suillus luteus* and *Amanita muscaria* for the phosphorus uptake of young pine plants (*Pinus silvestris*) was investigated using soluble and poorly soluble forms of labelled orthophosphate (P^{32}).

2. *Suillus luteus* always increased absorption of P^{32} compared with nonmycorrhizal controls while in experiments with soluble phosphate *Amanita* reduced phosphorus uptake. With iron phosphate and apatite this fungus reached or slightly exceeded the values of the controls.

3. In pot cultures both fungi similarly stimulated growth of pines, which may be explained by a mobilization of mineral nutrients from the walls of the clay pots.

4. When the microbiological competition in the soil is artificially stimulated by the addition of starch, phosphorus uptake of mycorrhizal and non-mycorrhizal plants decreases. A relative increase of phosphorus absorption by *Suillus luteus* was not found.

5. The addition of metabolic toxins to the rooting area depresses the phosphorus absorption of mycorrhizal and non-mycorrhizal plants.

References

Katznelson, H., Rouatt,, J. W. & Peterson E. A.: Mykorrhiza, Internat. Mykorrh. Symposium Weimar 1960, Fischer Jena, 273–278, 1963.

Kurmies, B.: Die Phosphorsäure *13* : 57–100, 1953.

Kuttner, T. & Lichtenstein, L. : J. biol. Chem. 95 : 661–670, 1932.

Meyer, L. & König E.: Landwirtschaftl. Forschung *13* : 7–24, 1960.

Rathje, W.: Z. f. Bodenk. u. Pflanzenernährung *28* : 129–159, 1942.

Ritter, G.: Die Bedeutung der Mykorrhiza für die Ernährung von Pinus silvestris L. Dissertation, Eberswalde 1963.

Santoro, T. & Casida, L. E.: Can. J. Microbiol. *8* : 43–48, 1962.